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Award Number: DAMD17-01-1-0525

TITLE: Detection of mitochondrial DNA mutations in mammary epithelial cells in nipple aspirate fluid

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REPORT DATE: September 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2002	Annual (1 Sep 2001 - 31 Aug 2002)

4. TITLE AND SUBTITLE

Detection of mitochondrial DNA mutations in mammary epithelial cells in nipple aspirate fluid

5. FUNDING NUMBER
DAMD17-01-1-0525

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)*** Because optimal methods have not been established for screening and early detection of breast cancer in premenopausal women, there is an urgent need for a variety of new approaches that can augment mammographic screening. We propose to study a new approach to early detection, based on mitochondrial DNA (mtDNA) mutation analysis of mammary epithelial cells shed into nipple aspirate fluid (NAF) and the Ductal Lavage (DL) fluid. The ability to detect these mutations in NAF and DL fluid, indicative of the early stages of breast cancer, would provide an important early detection approach that could complement conventional screening. Recent studies have shown the presence of several mtDNA mutations in a variety of tumor types, including colorectal, bladder, head and neck, and lung tumors. Furthermore, Sidransky's group has recently shown that these mitochondrial mutations are readily detectable in the bodily fluids, urine, sputum and bronchoalveolar lavage obtained from patients with bladder, head and neck, and lung tumors, respectively. They also showed that a mtDNA mutation at the microsatellite marker D310 is present in about 30% of breast cancers. We hypothesize that women with early-stage breast cancer may have mtDNA mutations in mammary epithelial cells shed into the NAF and DL fluid which can be used as a marker for screening and detection of early disease. Such mutations are expected to be absent or extremely infrequent in women with no evidence of breast cancer. In addition, we hypothesize that mtDNA mutations in NAF-derived cells are representative

14. SUBJECT TERMS	15. NUMBER OF PAGES
breast cancer, premenopausal women, DNA	7
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction

The optimal method for early detection of breast cancer in premenopausal women is not known. Currently, mammographic screening is the best available approach for early detection in the general population. However, mammography alone may not be sufficient in young women, for the following reasons: (a) effectiveness of mammography has not been established in women younger than 40 since younger women have more dense breast tissue, which reduces mammographic sensitivity (1), (b) tumor growth rates may be higher in younger women thereby necessitating frequent screening, and (c) carriers of some mutations (such as ataxia telangiectasia) may have increased sensitivity to radiation and conceivably could be harmed by frequent mammograms (2).

Genomic instability is characteristic of nearly all tumors (3). Accumulation of genetic and epigenetic changes is believed to play a pivotal role in each step of tumor progression. It is generally accepted that genetic instability occurs at an early stage in tumorigenesis (4, 5). Recent studies have also shown the presence of several mitochondrial DNA (mtDNA) mutations in a variety of tumor types, including breast, colorectal, bladder, head and neck, and lung tumors (6, 7, 8). A mtDNA mutation at the microsatellite marker D310, a mononucleotide repeat of 300–315 nucleotides which is located at the D-loop region of mitochondrial DNA and involved in the mtDNA replication process, was reported to be altered in about 30% of breast cancers. Mutations at this region are common in primary human tumors, including breast cancer and are likely to have functional relevance in tumor development (9). In addition, genetic aberrations have been detected in morphologically normal looking mammary epithelial cells surrounding breast cancer (10), suggesting that genetic changes might precede morphological alterations. Therefore, development of a method to detect genomic aberrations in mammary epithelial cells is an exciting opportunity for improving the detection of breast cancer at an early stage, particularly if performed on cells obtained through non-invasive or minimally-invasive approaches.

Nipple aspirate fluid (NAF) has been studied for many years as a non-invasive method to examine changes in breast biology and to identify women with high risk of breast cancer or preclinical disease (11). NAF is secreted continuously by the non-lactating breast and, in 50-70% of premenopausal women, it can be aspirated through ductal openings in the nipple using a simple, non-invasive pump. NAF is of interest because it has a relatively long retention time in the breast alveolar-ductal system where it accumulates exfoliated mammary epithelial cells (11, 12, 13). To date, most research has focused on conventional cytology to identify abnormal cells as an indicator of early progression toward breast cancer. However, NAF cytology alone is not sufficiently sensitive to identify the subgroup of women who are on a progression pathway that will lead to breast cancer (14). It is likely, however, that atypical epithelial cells destined to progress to cancer may have accumulated a number of premalignant genetic changes. Therefore, it may be possible to improve upon the sensitivity afforded by NAF cytology by examining these cells for genomic aberrations. Such an approach may provide not only a "snapshot" of the micro-environment where breast cancer originates, but also a realistic opportunity to improve the detection of breast tumors at their earliest stages. Our group is currently investigating new non-invasive approaches to early detection of breast cancer, based on studies of molecular markers of carcinogenesis detectable in the nipple

aspirate fluid (NAF). However, a major hurdle impeding the success of our assays is the low cellularity and low volume of NAF samples. The typical NAF sample from a woman with no breast abnormalities is about 10 μl in volume and contains fewer than 10 ductal epithelial cells[11]. One very promising new approach to overcoming these two issues has recently been designed and tested: Breast Ductal Lavage.

Ductal lavage (15) is a new, minimally invasive technique developed to evaluate the ductal fluid and cells. A small catheter is inserted into the duct. Saline is instilled and the breast is massaged to dislodge cellular material lining the duct. The fluid is then collected for analysis. In a recent study (15), ductal lavage was performed on greater than 500 high-risk women at 19 breast cancer centers. Atypical cells were seen in 17% of patients and suspicious or malignant cells were identified in 7% of patients. The lavage procedure also resulted in specimens sufficient for cellular analysis in a far greater number of patients than did nipple aspiration; in fact, ductal lavage produced an average of 40,000 cells per duct. This high yield of cells makes it much more likely that sufficient cells would exist for both cytologic analysis and for genetic studies. In addition, this procedure allows the physician to pinpoint the specific duct from which the abnormal cells originated, so that a specific area of the breast can be more thoroughly evaluated and more closely followed. Ductal lavage does have a number of limitations. The procedure is not successful in all patients (Reported success rate at the San Antonio meeting: 73%). If the procedure is successful, there is a percentage of specimens in which there is not enough cellular material for analysis (22% in the above study). In addition, ductal lavage is not possible in breasts that have been previously irradiated, and cannot usually be performed in patients who have had surgery near the nipple areolar complex. Lastly, ductal lavage carries a very small risk for infection; in the study mentioned above there were two reported cases of infection associated with the procedure. These were both successfully treated with oral antibiotics.

Body

In this project, we are investigating the possibility of using the lavage fluid and the nipple aspirate fluid to detect mitochondrial DNA mutations as an early sign of breast cancer and to determine the optimal method for detection of theses mutations. We will specifically set up the condition to detect mtDNA mutations at the microsatellite marker D310 in the DL and NAF fluids. As mentioned above, this mutation is present in over 30% of breast cancers and was discovered by Dr. David Sidransky's group in October 2001 (9). Our working hypothesis is that women with early-stage breast cancer may have mtDNA mutations in mammary epithelial cells shed into the nipple aspirate fluid (NAF) and the ductal lavage (DL) fluid which can be used as a marker for screening and detection of early disease. Such mutations are expected to be absent or extremely infrequent in women with no evidence of breast pathology (cancer). In addition, we hypothesize that mtDNA mutations in NAF and DL-derived cells are representative of those found in tumor tissue in the same breast.

During the first year of this project we have actively proceeded to achieve the groundwork for the success of this study. Because of the extensive experience of Dr. Sidransky's lab with mtDNA mutation studies, we have initiated a collaboration with his

group to set up an assay for the detection of mutations at the D310 marker in the DL and the NAF fluids. In addition we have started accruing DL and NAF samples to be tested.

Collaboration with Dr. Sidransky's group:
We have initiated an active collaboration with Dr. Sidransky and his group to study mtDNA mutations. As part of this collaboration, Dr. Luciane Cavalli, a post-doctoral fellow in my lab, spent time in Dr. Sidransky's lab at Johns Hopkins University School of Medicine in Baltimore, MD and worked closely with his group to learn how to perform the mutation analysis of the D310 marker. She subsequently sat up the assay in our lab and continues to interact with the group at Hopkins.

The assay is performed as follows:
The amplification and analysis of the mitochondrial microsatellite marker D310 is performed using a PCR-based assay as descibed by Parrella et al, 2001. Briefly, the DNA is amplified using a radioactively labeled forward primer (5'ACAATTGAATGTCTGCACAGCCACTT-3') and reverse primer (5'GGCAGAGATGTGTTTAAGTGCTG-3'). The PCR conditions are: 95°C for 2 minutes, 35 cycles of 95°C for 1 minute, 60°C for 1 minute, 72°C for 1 minute, and 72°C for 5 minutes. The PCR products is visualized using a 6% denaturing polyacrilamide gel. Currently, the conditions have been well established for assay to work on DNA prepared from NAF and DL in addition to blood.

Patient accrual:
We have obtained NAF and DL from 11 patient which are ready to be evaluated.

Key Research Accomplishments:

-Optimized conditions to detect mtDNA mutations in the D310 marker in DL and NAF.
-Collect 11 NAF and DL specimens for testing.Accrued 11 patients with *BRCA 1/2* mutations who underwent mastectomy and/or prophylactic mastectomy.

Reportable outcomes: N/A

Conclusions:

In this first year we have established the groundwork for the success of this project. We have initiated a collaboration with Dr. Sidransky's group at Johns Hopkins University and optimized the conditions to evaluate DL and NAF specimens for evidence of mtDNA mutations.

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